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Original article

# Assessment of serum levels of allergen-specific immunoglobulin E in different seasons and breeds in healthy horses

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## Abstract

The present study was designed to assess specific IgE towards environment allergens in 42 healthy horses. Determination of this immunoglobulin in serum serve as diagnostic tools in allergic diseases to improve efficacy of the treatment and proper allergen selection to specific immunotherapy. Serum levels of allergen specific IgE were measured with equine monoclonal antibody, using 15 individual and 5 mix allergens in North European Panel. The study revealed season dependent increased levels of allergen specific IgE in normal horses. It is noteworthy that healthy horses show high percentage of positive reactions, most commonly towards to domestic mites *D. farinae* (80%), *D. pteronyssinus* (35.71%) and storage mites *T. putrescentiae* (42.86%), *Acarus siro* (40.48%). These allergens play an important role in equine, canine and feline atopic dermatitis. We also demonstrated high IgE levels in the group of horse specific insect allergens. *Tabanus* sp. (35.71%), *Culicoides* sp. (28.57%) and *Simulium* sp. (26.19%) were the most frequent insect positive reaction allergens. No positive reactions in all groups of allergens were found in winter season, low and merely detectable levels of antibodies have been found relating to *D. farianae* and *T. putrescentiae* allergen. We observed elevated mould-IgE levels in horses that live in stables, while outdoor living horses showed very low levels. Amongst all positive reactions we observed only weak and moderate reactions but no strong positive reactions were found. No significant differences were observed between three breeds of horses with the exception of moulds and *D. pteronyssinus* allergens.

**Key words:** allergen specific IgE, healthy horse, seasons, immunology, allergens

## Introduction

Different serological assays are available to measure serum levels of allergen-specific IgE antibodies and can provide supportive evidence for a diagnosis of equine allergic dermatitis associated with environmental allergens (moulds, tree, weed and grass pollens, hay dust mites, insect saliva) (Kalina et al. 2003, Roque et al. 2011). These tests are used to identify causative allergens for avoidance and specific immunotherapy in animals with clinically diagnosed atopic dermatitis (AD) (Roque et al. 2011) or insect hypersensitivity (IH) (Hellberg et al. 2006). Type-I hypersensitivities are characterized by develop immediate skin reaction after allergen exposure resulting in activation of B-cells and production of allergen-specific IgE. This type of immunoglobulin mediated pathological reactions, binding to high-affinity IgE-receptors (FcεRI) on mast cells, induce inflammatory reactions by secreting chemical mediators. This interaction between IgE and the FcεRI receptor plays a key role in allergic inflammatory responses induced by mast cell degranulation, leading to pruritus and urticaria in horses (Wagner et al. 2006). Management strategies in such longlife chronic recurrent diseases include allergen specific immunotherapy based on *in vivo* intradermal test or *in vitro* IgE level detection serum test. However, several studies have questioned the reliability and reproducibility of some commercial serological allergy tests because of incidence of positive reactions in healthy humans and animals. Allergen-specific IgE serum concentrations may wane with lack of exposure, leading to negative allergen specific immunotherapy results in the off-season (Petersen and Schott 2009).

Studies also revealed sensitization to allergen with important role of IgE or IgG (T) in clinically healthy horses of different age groups (Wilson et al. 2001, Wagner et al. 2009). Genetic predisposition within breeds is expected to breed effect on the insect hypersensitivity reactions (Steinman et al. 2003). Also, horses of various breeds were compared and significant increase in serum levels of total IgE was found in horses suffering from skin hypersensitivity compared to healthy Icelandic horses but not for sera from healthy and affected horses of other breeds (Wilson et al. 2006).

Specific IgE levels are not always detectable in the sera of some atopic humans and animals. This phenomenon can indicate that some cases of canine AD are not IgE mediated (atopic-like dermatitis). The significance of circulating allergen-specific IgE in the pathogenesis of atopic dermatitis remains not completely clear.

Our aim was to assess allergen-specific IgE levels in healthy horses in different breeds during two seasons (winter, spring). Quantitative estimation of IgE level in healthy animals allows to better interpretation of diagnostic serologic test results and immunologic status of allergic horses. Comparison of specific IgE level in various groups of allergens in winter and spring seasons also allows to determine of adequate time of serologic test performing. This is the first report about allergology status of horses in Poland.

## Materials and Methods

The study was performed on clinically healthy horses without signs of skin or respiratory diseases or other allergic conditions before or during this study. Groups were composed of 42 horses of the Malopolski breed (n=18), Primitive Polish Horses (n=12) and Pony (n=12) belonging to three studfarms. 52.38% of horses in all the groups were females and 47.62% were males. The animals ranged in age from 2 to 14 years (with a mean age of 7.74 years). Horses were kept loose in group housing stables during the winter and during the summer at pasture and in stables.

Sample collection and IgE measurement were perform in spring (May) and winter (January) of 2011. Five ml of peripheral blood were collected by jugular vein venipuncture from all the horses. Serum allergen-specific IgE level was determined using a mon-

Table 1. Allergens used in specific IgE level determination test.

| Allergens                                    |  |                                     |
|--|--|-------------------------------------|
| Mites  | 1. <i>Dermatophagoides farinae</i>             |                                     |
|  | 2. <i>Dermatophagoides pteronyssinus</i>       |                                     |
|  | 3. <i>Lepidoglyphus destructor</i>             |                                     |
|  | 4. <i>Acarus siro</i>                          |                                     |
|  | 5. <i>Tyrophagus putrescentiae</i>             |                                     |
| Moulds                                       | 6. <i>Apergillus/Penicilium</i> sp.            |                                     |
| Bacteria                                     | 7. <i>Thermoactinomyces/Micropolyspora</i> sp. |                                     |
| Plant pollens                                | 8. Ragweed ( <i>Ambrosia</i> sp.)              |                                     |
|  | 9. Birch/Alder/Hazel                           |                                     |
|  | 10. Platan/Oak/Olive tree                      |                                     |
|  | 11. Cultivated rye                             |                                     |
|  | 12. Mixed grasses                              |                                     |
|  | 13. Rigwort                                    |                                     |
|  | 14. Mugwort                                    |                                     |
|  | 15. Colza                                      |                                     |
|  | Insects  | 16. Horsefly ( <i>Tabanus</i> spp.) |
|  |  | 17. <i>Culicoides nub.</i>          |
| 18. Mosquito                                 |  |                                     |
| 19. Blackfly                                 |  |                                     |
| 20. Stablefly ( <i>Stomoxys calcitrans</i> ) |  |                                     |

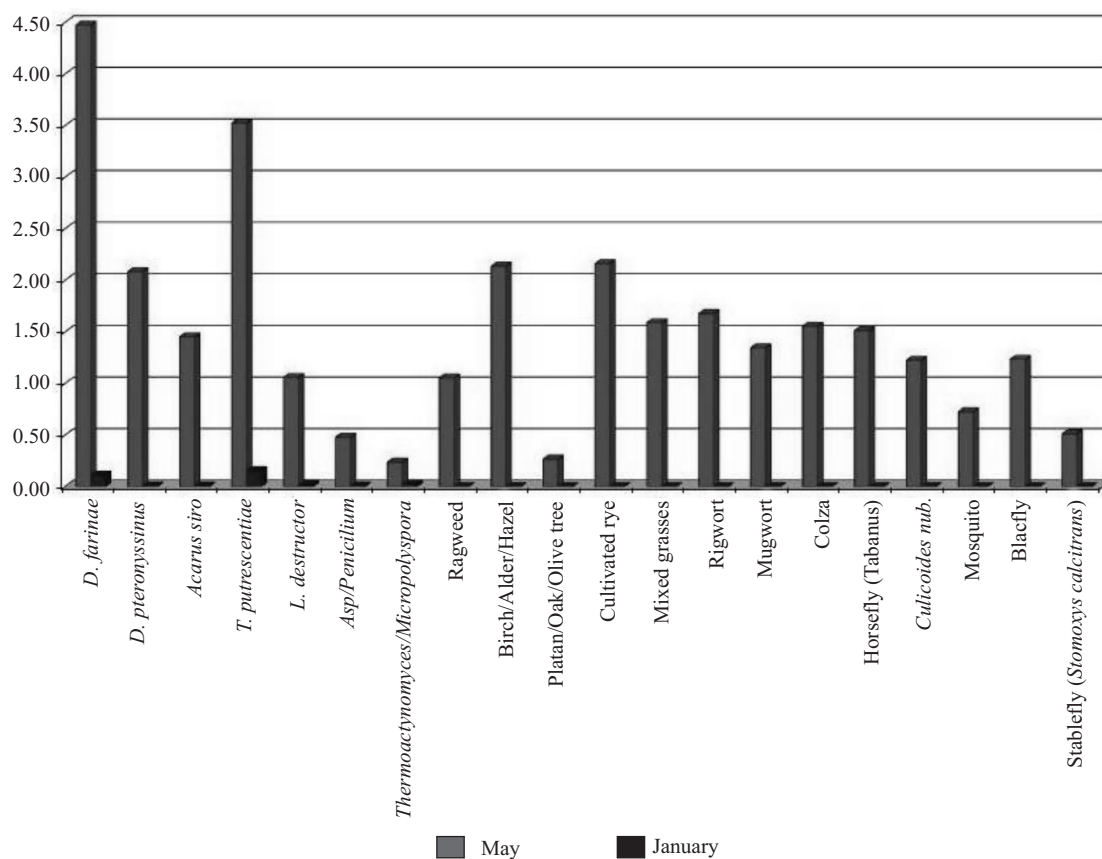


Fig. 1. Mean values of allergen-specific IgE concentrations (kU/l) in sera of Primitive Polish Horses (n=12) in May and January.

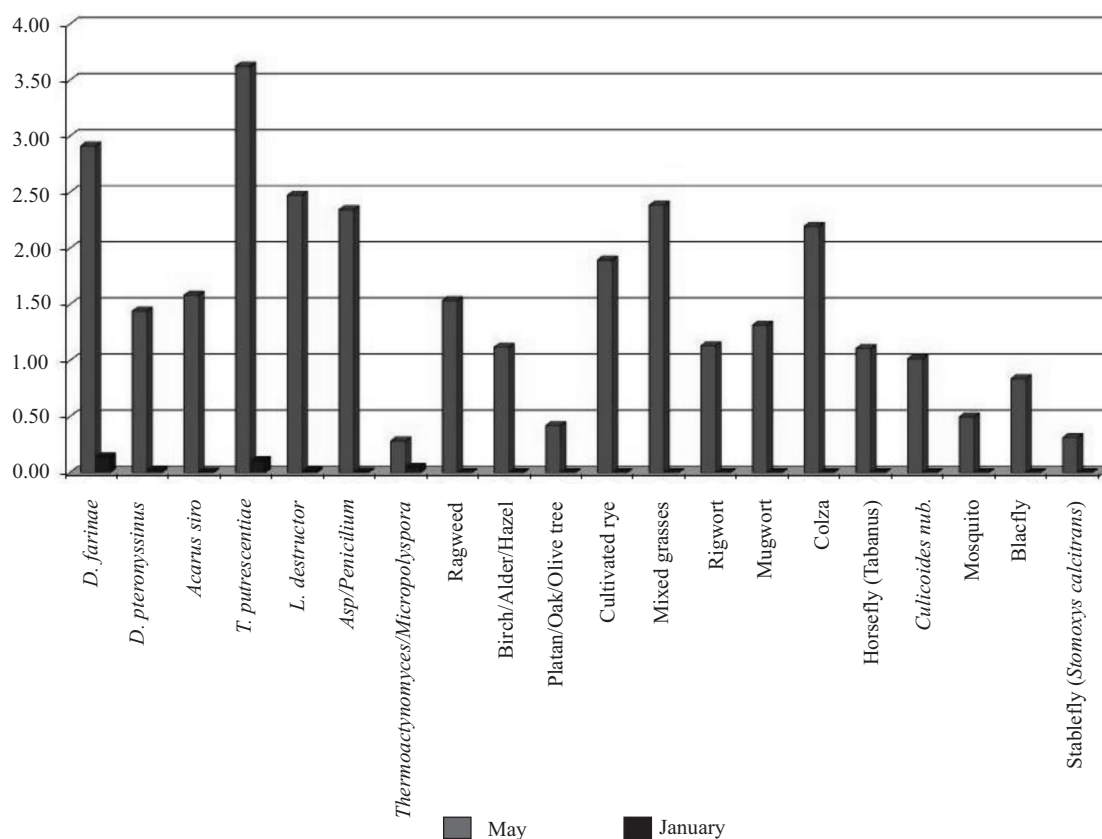


Fig. 2. Mean values of allergen-specific IgE concentrations (kU/l) in sera of pony (n=12) in May and January.

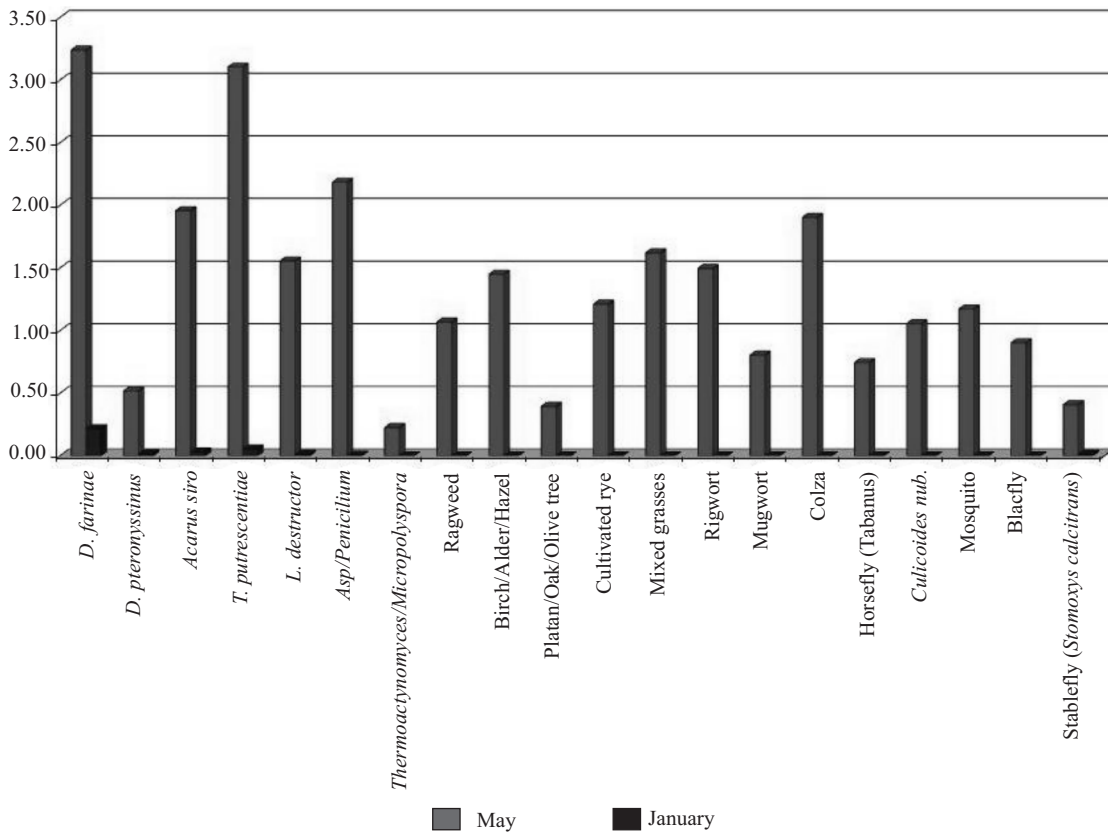


Fig. 3. Mean values of allergen-specific IgE concentrations (kU/l) in sera of Malopolski breed (n=18) horses in May and January.

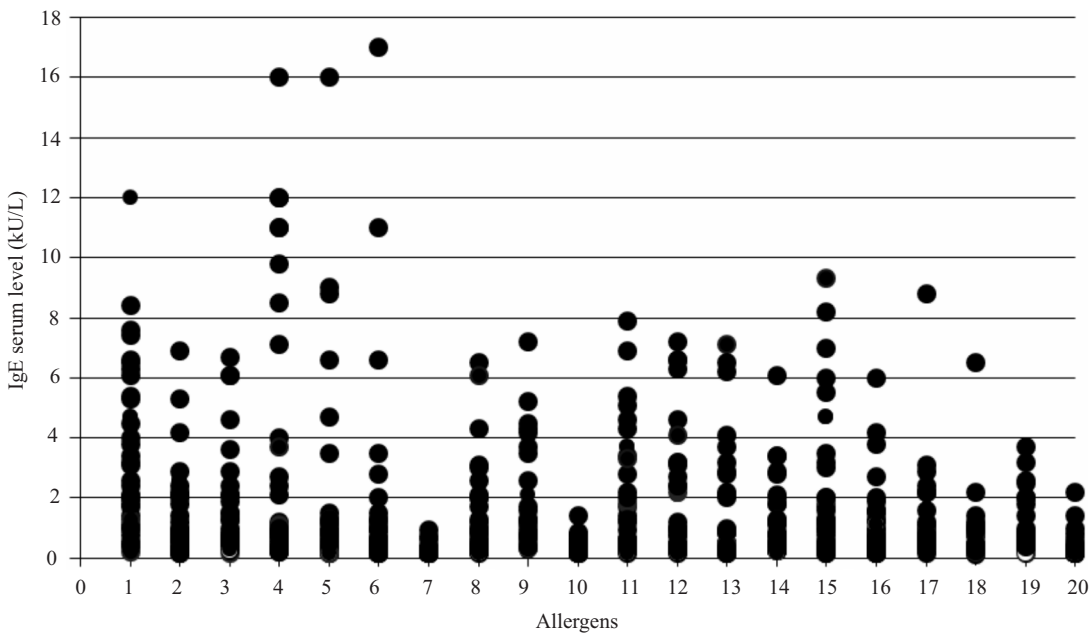


Fig. 4. Allergen-specific IgE serum concentrations determined in all groups of healthy horses (n=42) in May.

oclonal anti- IgE antibody (Polycheck Allergie NF Horse Panel, BioCheck GmbH) according to the manufacturer’s instructions. The North Europe Allergen panel was composed of 15 individual and 5 mix allergens (Table 1).

The statistical analysis was performed with STATISTICA 6.0 for Windows (StatSoft Inc.). The analysis was conducted by the Mann-Whitney U test at P-values of  $p < 0.05$ .

All investigations involving the use of animals

were approved by the Local Ethics Committee. No conflicts of interest have been declared.

## Results

The results of allergen-specific IgE levels in three breed of healthy horses were depicted in Fig. 1,2,3,4. In accordance with the guidelines for the interpretation of the results provided by the manufacturer of the test negative reactions ( $< 1$  kU/l), low-value reaction (1.0-2.0 kU/l), positive (2.0-20 kU/l) and strongly positive ( $> 20$  kU/l) were considered.

The highest IgE mean values were found in a group of Primitive Polish Horses in relation to *Dermatophagoides farinae* (4.84 kU/l; within range 1.1-8.4 kU/l), in Malopolski breed (3.24 kU/l; 0.22-12 kU/l) and in a group of ponies (2.91 kU/l; 1.0-6.3 kU/l). Moderately high IgE mean values were obtained in horses in relation to *Tyrophagus putrescentiae* in ponies (3.63 kU/l; 0.24-16 kU/l), in Primitive Polish Horses (3.52 kU/l; 3.8-8.5 kU/l), and in the Malopolski breed (3.10 kU/l; 0.24-12 kU/l). The lowest mean values in a group of dust mite allergens were found in Malopolski breed in relation to *Dermatophagoides pteronyssinus* (0.56 kU/l; 0.15-2 kU/l). In the group of all horses, positive reactions were found in relations to the *D. farinae* (80.95%), less often to other mites *T. putrescentiae* (42.86%), *Acarus siro* (40.48%), *D. pteronyssinus* (35.71%) and *Lepidoglyphus destructor* (33.3%). In the group of all horses, values of mite allergens ranged from 0.22 to 16 kU/l, and the positive reactions were found in 38.1%. In any event, the strong positive response was not observed.

The highest values of levels of the insect-specific IgE were obtained from the Primitive Polish Horses to the *Tabanus sp.* allergen (1.51 kU/l; in range 0.22-4.2 kU/l), the lowest to *Stomoxys calcitrans* in ponies (0.31 kU/l; 0.16-0.84 kU/l). In group of all horses 35.71% positive reactions were observed for the *Tabanus sp.*, 28.57% for *Culicoides sp.*, 26.19% for *Simulium sp.*, 19.4% for mosquito and rarely 9.52% for *Stomoxys calcitrans*. Positive reactions towards to insect allergen were found in 23.81% of all horses. Values of specific IgE of all the groups of horses were ranged from 0.15 to 8.8 kU/l. No strongly positive reactions were found.

Most frequently, the highest reported mean values of pollen-specific IgE were observed against rye grass allergen (2.39; in range 0.24-7 kU/l) and colza allergen (2.20; within 0.36-8.2 kU/l) in ponies. Also, high levels of antibodies were found in relation to birch, alder and hazel pollens (2.14 kU/l; 0.4-7.0 kU/l). Similar levels of antibodies were found in relation to grass mix pollens (2.39 kU/l; 0.25-7.2 kU/l). In all the groups of

horses most positive reactions were found for the colza pollen allergen (52%) and grass mix (48%) and the rarest for *Ambrosia sp.* (33.3%).

In the group of mould allergens significant differences were found between different breeds of horses. High mean values were in ponies (2.35 kU/l; in range 0.19-17 kU/l) and Malopolski breed (2.18 kU/l; 0.15-11 kU/l). Very low values were observed in Primitive Polish Horses (0.48 kU/l; 0.15-1.2 kU/l). In all the investigated groups positive reactions on the moulds occurred in 28.6% of horses.

The lowest mean values for IgE were demonstrated for *Thermoactinomyces sp.* *Micropolyspora sp.* allergens (0.25; in range 0.23 to 0.29 kU/l) and in any case there was no positive response.

The mean levels of allergen-specific IgE obtained in May were significantly different from those observed in January. The differences occurred for all groups of horses and for all groups of allergens. Rarely, detectable levels of IgE were found in winter and only in cases relating to *D. farianae* and *T. putrescentiae* allergen. No positive reactions were found in winter season.

No statistically significant differences between horse breeds were detected in the values of individual levels of allergens, with the exception of allergen *D. pteronyssinus*. In this case, we demonstrated differences between Primitive Polish Horses and Malopolski breed ( $p = 0.000479$ ), as well as between Primitive Polish Horses and ponies ( $p = 0.002471$ ).

## Discussion

Allergy testing is an important component of the diagnostic procedure to identify specific allergens. Although history and clinical diagnostic play paramount role in diagnosis of allergy, serological tests confirm sensitization and help in treatment recommendations during specific immunotherapy. Currently, allergen-specific IgE antibody assays are designed to detect serum levels of circulating IgE. These *in vitro* tests are based on immunoassay principles using polyclonal or monoclonal antibodies (Morgan et al. 2007).

In the present study we measured allergen specific IgE serum levels using coated allergens and monoclonal antibodies directed against equine IgE. Allergy panel contains of 15 single and 5 mix allergens (house dust mites, storage mites, insects, moulds and grass, weed, tree pollens). Careful observation was conducted during two years before blood sampling and only the animals without history of skin or respiratory tract disease were included to the clinically healthy group. Serum samples were collected in winter (January) and spring (May) from horses belonging to

three breeds. Our observations confirmed increased levels of allergen-specific IgE in clinically healthy horses against mite, insect, mould and pollen allergens. This condition occurs in spring season in contrast to winter time when IgE levels were almost undetectable with the exception of house and stable mites. All "spring positive reactions" were of mild (0.15-2 kU/l) or moderate intensity (2-20 kU/l), there were no strong reactions (> 20 kU/l). Other studies have also reported induction of positive reaction (PR) in healthy horses. 35% of the Icelandic horses gave a positive reactions with at least one insect extract. In our study 28.71% horses demonstrated PR, but off-season insect allergen-specific IgE were undetectable. Meulenbroeks et al. (2013) observed no difference between the levels of specific IgE serum titers obtained during off-season time and in the season in horse suffering from insect bite hypersensitivity. These observations could indicate continuous production of IgE by plasma cells without B cells reactivation in allergic horses. Allergen specific IgE levels increased temporarily during contact with allergens, then decreased after allergy season in the normal animals. Meulenbroeks et al. also maintain that circulating allergen-specific IgE levels do not reflect the amount of mast cell- or eosinophil-bound IgE what leads to inflammatory response and clinical symptoms of allergy. Detectable allergen specific-IgE levels do not indicate allergy in horses but only a periodic seasonal reaction on environmental allergens. Moreover, besides allergen-specific immunoglobulin E class, IgG subclass can play a role in hypersensitivity in horses (Wagner et al. 2006) and exists in equine serum in much higher concentrations (Morgan et al. 2007). Prevalence of high levels of specific IgE in healthy animals can exist in sub-clinical form of an allergic disease. However, none of the animals included in this study had allergic manifestations in the following years.

A study by Frey et al. (2008) conducted on healthy horses showed a significant increase in serum levels of specific IgE to house dust mites (32%) and pollens (27%). Our study revealed higher percentage of positive reaction to house (36-81%) and storage mites (33-43%) with detectable levels in winter. Although we confirmed detectable levels of mite-specific IgE in winter (0.15-1 kU/l), no positive reactions were recorded. Lower humidity and temperature could cause decrease in mite population outdoors as well as in the stables. Mite allergens are considered a major group of allergens causing atopic dermatitis in humans, dogs and cats. The high percentage of positive reactions described above indicates that mites are the main group of allergens also in horses. In our study, positive reactions to mites were the most frequent amongst all groups of allergens.

Significantly high level of pollen-specific IgE were observed, mainly towards colza and grass mix, 52% and 48% positive reactions respectively. Such a high percentage of PR could be explained by the season of pollination of these plants during the blood collection time.

Interestingly, in contrast to other studies (Frey et al. 2008), our investigations revealed an increase in mould-specific IgE levels in two groups of horses. Values of IgE levels towards moulds were comparable to grass pollen allergens in Malopolski breed and pony. Primitive Polish Horses had significantly lower levels of mould IgE compared to those determined in the pony and Malopolski breed. However these distinctions could result from husbandry factors and different environmental conditions. Similarly to the previously published findings by Eder et al. (2001) we observed important role of the environmental factor with regard to serum IgE levels against moulds *Aspergillus* and *Alternaria* allergens. Elevated allergen-specific IgE levels were observed in the animals residing in stables, while the horses living outdoor showed very low IgE levels against moulds.

In conclusion, the results of this study suggest that allergen specific-IgE positive reactions are commonly found in healthy horses. The levels of specific IgE are season-dependent. There is no breed influence on specific IgE level. Further investigations are needed to compare specific IgE in healthy and allergic horses using the monoclonal antibody test.

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